IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

December 13, 2005

In Re application of:	Couto, *et al.*
Serial No:	10/635,117
Filed:	08/06/03
For:	**METHODS OF TANGENTIAL FLOW FILTRATION AND AN APPARATUS THEREFORE**
Art Unit:	1653
Examiner:	Robert B. Mondesi
Attorney Docket Number:	GTC – 207

DECLARATION OF MARK A. PERREAULT

Commissioner Patents
PO Box 1450
Alexandria, VA 22313-14590

I, Mark Perreault hereby declare and say as follows:

BACKGROUND OF DECLARANT

1. I received my Bachelor in Chemistry Degree from the University of Massachusetts in 1994. A partial list of professional accomplishments is attached hereto as Exhibit A.

2. I have been employed by GTC Biotherapeutics Inc. ("GTC") of Framingham, Massachusetts, since 2002. From the time I was hired until the present I have been engaged in the improvement of existing Filtration processes and Analytical Systems for biopharmaceutical production and purification.

3. I worked with the inventors of the above referenced patent application, am aware of its contents and teachings and have signed this declaration on my own behalf.

4. I am an inventor of similar technologies for GTC, with other applications currently under examination in front of the United States Patent and Trademark Office.

NEED FOR IMPROVEMENT IN METHODOLOGY

5. During our research, and prior to the filing of the above referenced patent, it was determined by GTC personnel that to reduce the costs of production and to prepare for large scale production of biopharmaceuticals from the milk of transgenic animals improvements had to be made to existing bioseparation processes. Moreover, existing teachings relative to protein purification were inadequate relative to the processing of transgenic milk.

6. GTC personnel, including myself and the inventors of the above referenced application, realized before the filing of the Couto et al., 10/635,117 application that there was a need in the art for improvement in existing protocols for selectively separating molecules such as peptides, polypeptides, and non-peptidyl compounds from other molecules using a process that increases yield, is less expensive and is less denaturing. In particular, there was a need for purification techniques to allow the separation of a molecule of interest from a fermentation broth as utilized in cell culture or a milk feedstream produced by a transgenic mammal. This need was not answered by the prior art, including van Reis et al.

7. Purifying a recombinant protein from milk is technically complex and expensive. The purification process must be reproducible, involving as few labor-intensive steps as possible, and maximize the yield of the target protein as measured by its biological activity. An ideal purification process optimizes yield, keeping manufacturing costs low. Cow milk is about 87% water, 4–5% fat, 5% carbohydrate, and 3–4% protein. Goat's milk and sheep's milk have lower fat content but higher protein content. Lactose is the major carbohydrate in the milk of most species - and the least variable component of milk. The fat component is a complex mixture of lipids secreted as globules primarily composed of a triglyceride surrounded by a lipid bilayer membrane, which helps to stabilize those fat globules in an emulsion within the aqueous environment of milk. More

than 95% of total milk lipids are in the form of globules ranging from 0.1 to 15 μm in diameter. These liquid fat droplets are covered by a thin membrane, 8–10 nm thick, with properties completely different from both milk fat and plasma. The native fat globule membrane (FGM) is an apical plasma membrane of the secretory cell that continually envelopes the lipid droplets as they pass into the lumen. The major components of that native FGM, therefore, are protein and phospholipids. The major milk protein is casein. The principal casein fractions are (s1) and (s2) caseins. The distinguishing property of all caseins is their low solubility at approximately pH 4.6. A common compositional factor is that caseins are conjugated proteins, most with phosphate group(s) esterified to serine residues.

8. Milk chemistry and composition is a very unique and different starting material from a separation and filtration point of view. As provided above, milk is a unique feedstream that is comprised of > 6% solids and contains high levels of protein (~ 70 grams per liter) as well as many different kinds of fat. Fat globules and casein micelles are both greater than micron sized particles that cause novel issues filtration efforts and filter cleaning. In addition, calcium, phosphate and many other minerals are found in milk that makes the overall chemistry extremely complex. This complexity meant that unique and novel analytic processes had to be developed to contain costs and insure a high quality pharmaceutical grade product. This is what GTC accomplished with the above referenced application.

9. The development of the techniques taught in the referenced patent application provide a method for the accelerated processing of human therapeutic proteins, protein fragments, or antibodies from a variety of feedstreams, preferably from transgenic mammalian milk that improves upon the prior art. Specifically, the filtration technology developed and provided by the referenced patent application provides processes and parameters for the clarification, concentration and fractionation of the desired recombinant protein or other molecule of interest from the native components of milk or contaminants thereof. The resulting clarified bulk intermediate is a suitable feed material for traditional purification techniques such as chromatography which are used down stream from the tangential flow

filtration ("TFF") process to bring the product to a final pharmaceutical grade formulation and purity. This advance was important in making the entire industry as related to the transgenic animal production of biopharmaceuticals feasible from a long run commercial perspective.

Declarant further states that the information presented above, and in the patent application referenced above, clearly demonstrates the following facts:

FILTRATION AND BIOSEPARATION

10. With regard to the question of particles greater than 0.1μm it should be noted that according to the instant invention the specification teaches and the current claims recite that GTC uses membranes to separate particles from small molecules in a milk feedstream; these particles are greater than 0.1 um (such as, fat globules, casein micelles, and cells). The membrane is used to perform a separation between unwanted contaminants and the valuable transgenic proteins expressed in the milk. Each of these unwanted particles are well-known components of milk and their size is well documented in the art, each pose part of the problem in developing filtration methodologies and product harvesting in good yield.

Fat globules –	0.1 – 20um
Casein micelles -	0.05 – 0.6um
Cells -	10 – 100um

11. Milk is clearly embodied in the patent application and may be found as a reference in claim 8 of the original application, as well as various other supports in the specification. [Original Specification: para. 4, page 2; para 14, p. 6; para 17, p. 6; para 18, p. 6; Fig. 5; Fig. 9].

12. The membrane used for the removal of the described milk particles according to the invention, and consequently providing the limits of filtration size as recited in the claims, is from the methods section of the specification. The unit used by the inventors was a

filter that was ceramic based and had a nominal pore size of 0.2um. A 0.2um microporous membrane will retain particles in the "micron" range from 0.1um to 10 um. The membrane used for the clarification experiments were indeed used to separate these particles from the desired product. Specific references to the membrane used may be found in the materials and methods section of the application, and is therefore implicit in the data provided. Application References: [Original Specification: page 10 – Definition of Clarification; para 42, p. 13 – recitation of Clarification steps; Fig. 1; para 45, p. 15 – Milk as feedstream with what that means in terms of particle size to those working in the field; para 71, p. 21 ceramic filter pore size; para 74, p. 22; para 78, p. 24; para 81, p. 25 – membrane pore size].

13. As is known in the microfiltration field, beyond a critical concentration and pressure the fluid feedstream forms a separate phase where the Brownian motion of the solute molecules is "frozen". This phase transition point differentiates between the true solution behavior (disordered phase) and a cake type behavior (ordered phase). In classical studies on ultrafiltration, the solute concentration at this point is often termed the 'gel concentration.' In terms of microfiltration, an increase of one or more of the four operating parameters (TMP, pore size, pore size distribution and feedstream concentration) results in higher pore blocking propensity. The influences of these four operating parameters on the cake property are different, but each are involved during any microfiltering process. Scientists and inventors must pay attention to each of them during every experiment if they are to optimize their filtration processes – as was done in the current specification and as recited in the claims.

SUPPORT FOR CLAIM AMENDMENTS AND RECITED OPERATING RANGE

14. It is important to note that the experimental microfiltration data found in the original patent application was used to support the unique nature of the invention. The data developed from an extensive amount of experimentation was organized into a series of graphs and used to determine the ideal operating conditions of a microfiltration

membrane. These conditions were then used for the separation of milk components from the transgenic protein expressed in milk. The data shows the optimal cross-flow rate, operating temperature, concentration factor, and the transmembrane pressure (TMP) for the separation. Most importantly, the TMP was found to be optimal at approximately 15psig for the process described. As has been noted by Applicant's counsel, however, when the data was initially presented in this manner it did not clearly demonstrate the differences in operating conditions between the ones used by van Reis et al., and the above referenced GTC application. This was because the inventors presented their data on graphs in one way and van Reis another. When the data was plotted again for the benefit of the Examiner of this case before the USPTO, in a manner consistent with the methods of van Reis, the distinct differences between the existing patents of van Reis and the GTC invention became clear.

15. All the data provided in the specification is represented in the amended claims and is derived directly from the original application, citations to support the operation of the instant invention above 100% transition point flux parameters can be found in the application as filed. Application References: [All from Original Specification: para 87, p. 28 – Optimal Parameters – above van Reis; Graphs E and F p. 30; para 79, p. 24 – where the current invention "operates"; para 105, p. 42 optimum flow velocity – above van Reis; para 90, p. 32 – where the current invention TMP "must" be run above van Reis' parameters; para 105, p. 42 – optimal flux of IgG1; Chart. Page 36 – optimal operating parameters in a different region of the comparative curve relative to van Reis; para 117, p. 48 directing how to adjust the parameters of the invention in the region in 100% of van Reis].

16. It should be noted that Graphs B and F of the current application demonstrate the relationship between transmembrane pressure (TMP) and Mass Flux (GMH), a measure of liquid flux and productivity. When simplifying this graph, productivity was not graphed simultaneously, leaving TMP and liquid flux (LMH). The conclusion which may be made from this information is that the original application, and the amended claims, show optimal transmembrane pressure above the transition point of the curve – above

100%. This is an important piece of information as it further differentiates GTC's invention from the cited prior art.

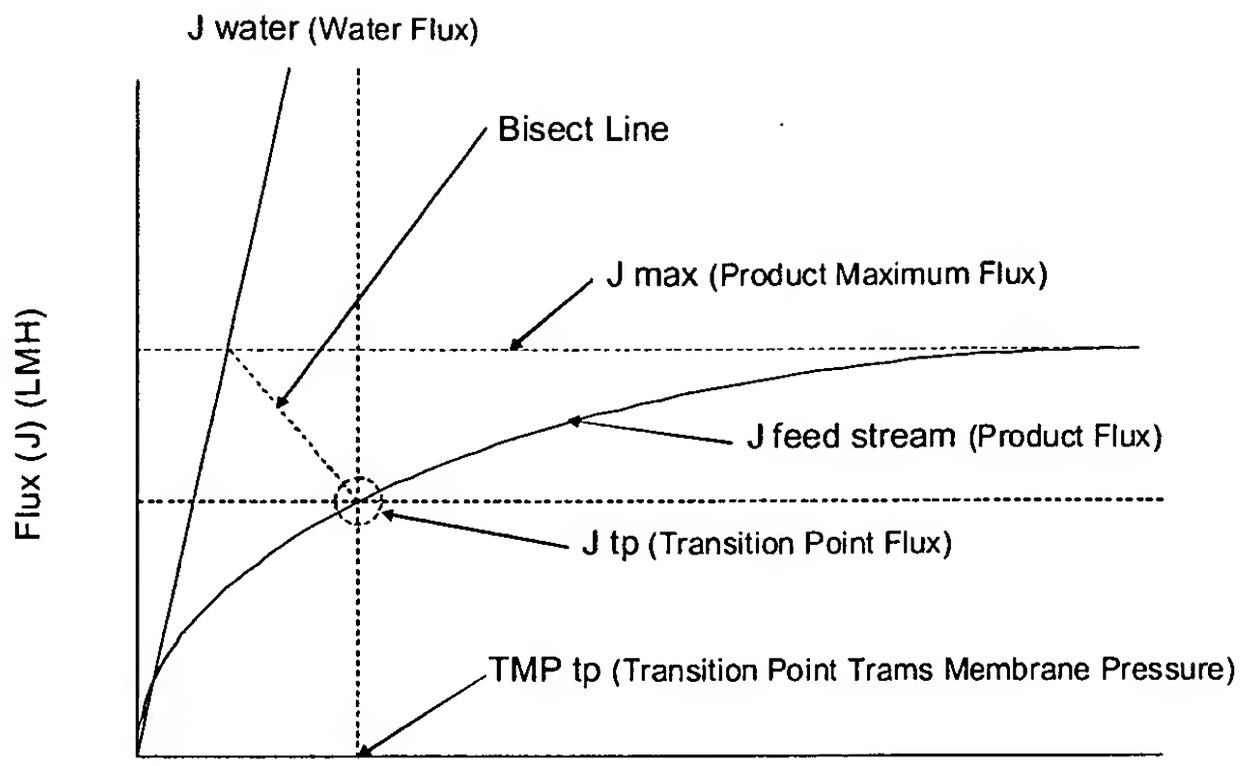
17. The data of the application referenced above shows the optimal cross-flow rate, operating temperature, concentration factor, and the transmembrane pressure (TMP) for the separation. Most importantly, the TMP was found to be optimal at approximately 15psig for the process described (Fig. 7), and has been presented during the prosecution of the application. This optimal TMP, of 15 psig, is higher than the transition point pressure of 2.5 psig supported by Table D. Unfortunately, when the data was presented in this manner it did not clearly demonstrate the differences in operating conditions between the ones used by van Reis and GTC. Again, this was because the inventors presented their data on graphs in one way and van Reis another. Application References: [All from Original Specification: Figs. 1, 3 and 7; para 87, p. 28 – Optimal Parameters – above van Reis; Graphs B, E and F ; para 79, p. 24 – where the current invention "operates"; para 117, p. 48 directing how to adjust the parameters of the invention in the region in 100% of van Reis].

VAN REIS et al., KONDO et al., AND/OR KUNIHAU et al.

18. After a review of the citations provided by the Examiner against the current claims it is my opinion that substantial differences remain. The difficulty in seeing this was primarily caused by the way in which the data was presented. When van Reis is plotted on the same data curves as the current invention it is clear that van Reis "operates" in a different fashion from the current invention and its corresponding claims. This provides for the foundation for saying that van Reis and the current invention are definitively different.

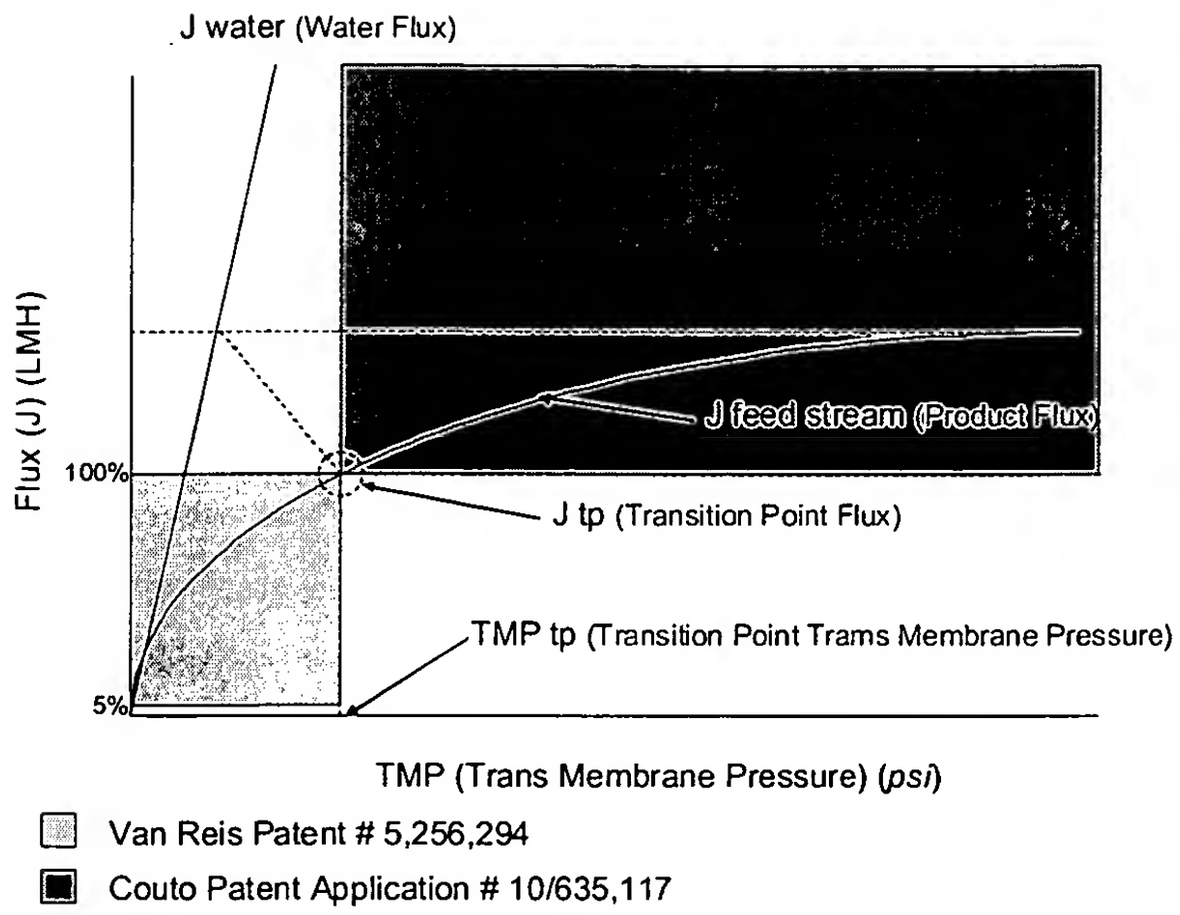
19. Typical data along this line is presented below. When compared to van Reis data it clearly demonstrates the difference between the existing prior art and the current invention. This is true of van Reis et al., Kondo et al., and Kunihau et al., as presented to Applicants by the Examiner in this case.

"Diagram # A"



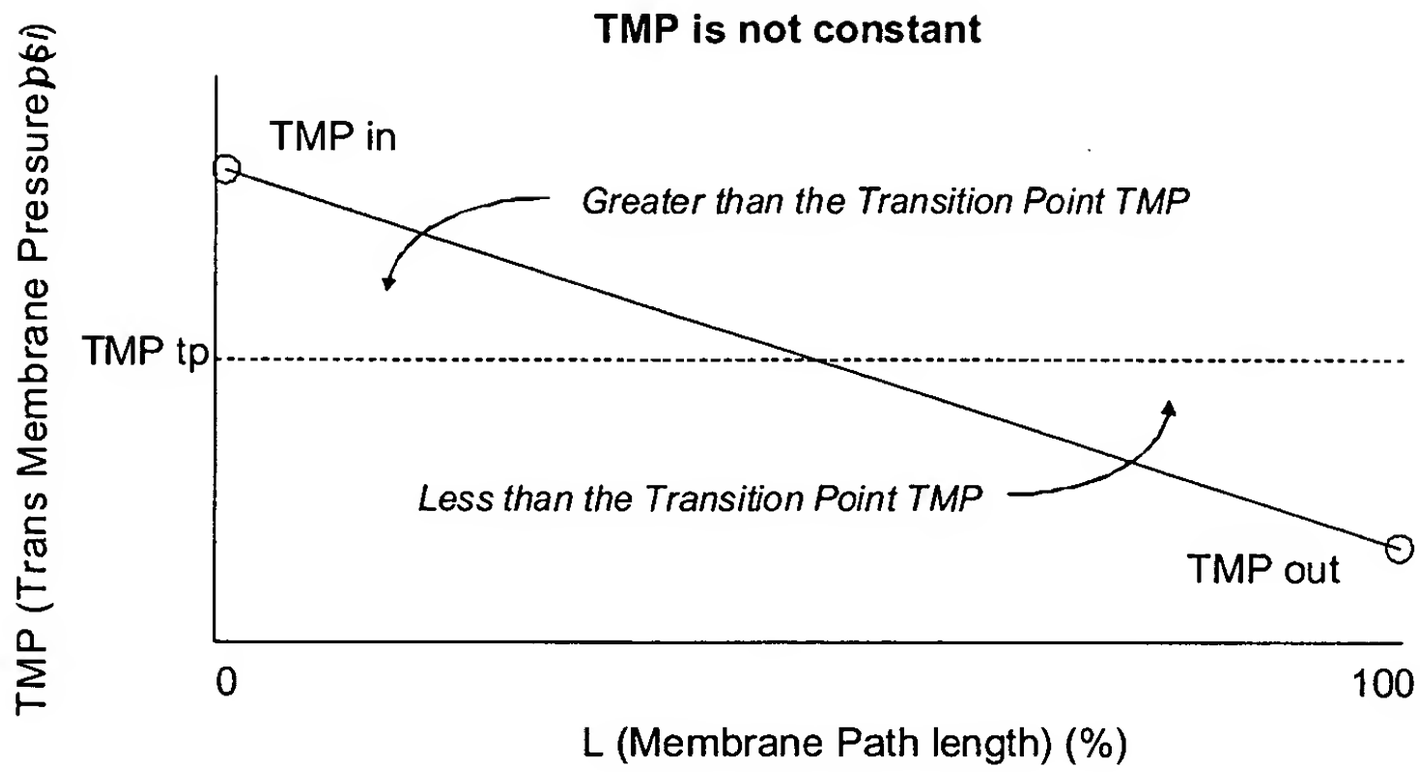
18. In Diagram # A, some of the terms for Claim # 1 of the referenced patent application are defined. With in a TMP vs. Flux graph, two major lines are drawn: the Water Flux (J water) and the Product Flux (J feed stream). The Product Flux curve defines a maximum product flux (J max) which intersects the water flux line. At this point a bisecting line is drawn from the intersection of the two lines (J water and J max) to the Product Flux curve. The point at which the bisect intersects the J feed stream curve is defined as the transition point flux (J tp). A line is dropped directly down to the X axis to define the transition point transmembrane pressure (TMP tp).

Diagram # B



19. In the Van Reis patent # 5,256,294, the zone that is in Claim #1 is the area in light blue in Diagram #B bound by 100% to 5% of the Jtp and the TMP tp vertical line. The current invention rests its amended claims in the area of Diagram #B in light green bound by a Flux greater than 100% of the Jtp and the TMP tp vertical line.

Diagram # C



20. In Diagram # C, additional terms for Claim #1 of the patent application are defined as per the specification. With in a Membrane path length vs. TMP graph, a major line is drawn from the membrane inlet TMP (TMP in) to membrane outlet TMP (TMP out). We previously defined on Diagram # A, a point of the X axis as the transition point transmembrane pressure (TMP tp). In Claim # 1 of the van Reis '294 the TMP is stated to be substantially constant and at a level no greater than the TMP tp vertical line. Respectfully, the instant patent application denotes the parameters in Diagram # C that are not constant and include TMPs at a level greater than the TMP tp vertical line. This data can also be illustrated in Diagram # D.

Table # D

	Membrane	Average TMP Inlet (psi)	Average TMP Outlet (psi)	TMP Transition Point (psi)
Atech	A	3.8	2.2	1.5
Corning	B	4.9	0.8	1.5
Orelis	C	4.4	1.9	2.5
Tami	D	5.2	0.8	4.0
Average		4.6	1.4	2.4

21. Studies conducted on the techniques of the invention reveal the following factors not present or suggested in any of the teachings of van Reis, with amendments these have grown to include:

a) usage of milk as a feedstream;

b) primary usage of ultrafiltration as opposed to microfiltration;

c) operation at entirely different flux level and pressure parameters; and,

d) dramatic differences in pressure variances.

22. The techniques of the TFF process taught by the referenced application concentrate the desired target molecule to a level suitable for optimal down stream purification and overall product stability. This concentrated product is then aseptically filtered to assure minimal bioburden and enhance stability of the product for extended periods of time. The bulk products produced by the Couto et al., invention will realize a purity between 65% and 85% and may contain components such as goat antibodies, whey proteins, and low levels of residual fat and casein. This partially purified product is an ideal starting feed material for conventional down stream chromatographic techniques.

23. Typical of the products that the teachings of the current application can be used to process are immunoglobulin molecules, including without limitation: IgG1, IgG4, IgM, IgA, Fc portions, fusion molecules containing a peptide or polypeptide joined to a

immunoglobulin fragment. Other proteins that can be processed by the current invention include recombinant proteins, endogenous proteins, fusion proteins, or biologically inactive proteins that can be later processed to restore biological function. Included among these processes, without limitation, are the proteins antithrombin III, human serum albumin, decorin, human alpha fetoprotein urokinase, and prolactin

THE TECHNIQUES ARE NOVEL

24. The methods of the above referenced application also provide a precise combination of filters and conditions that allow the optimization of the yield of molecules of interest from a given feedstream. In these methods the process parameters such as pH and temperature are precisely manipulated. These tangential-flow filtration processes for separating species such as particles and molecules by size, are selective for the species of interest, resulting in higher-fold purification.

25. It is my professional opinion that the processes and techniques taught in the above referenced patent application are novel, extremely useful and at variance with what went before relative to the production of pharmaceutical grade products from the milk of transgenic non-human mammals.

26. Moreover, I believe that the use of the techniques in the above referenced application are not only enabled by the specification as filed, but also support the amended claims now before the USPTO.

EXPLICATED METHODS HAVE UTILITY

27. The protocols provided in the referenced patent application are capable of utilizing tangential flow filtration (TFF), in a rapid and more efficient method for biomolecule separation than seen previously. The techniques of the current invention are also

applicable to a wide range of biological fields such as immunology, protein chemistry, molecular biology, biochemistry, and microbiology.

PROCESSES CLAIMED IN PATENT ARE ENABLED FOR INDUSTRIAL USE

28. The research results we gathered and our day-to-day experience demonstrates that the methods and processes claimed in the above referenced patent application allow for the production of bioactive biopharmaceuticals demonstrating significant activity against a plethora of pathologic conditions.

29. The above referenced application provided data from a large number of bioseparation and processing experiments more than sufficient to establish a reasonable expectation that the claimed methods and processes have a practical utility associated with the final production of biopharmaceutical compounds from transgenic milk or other biological feedstreams.

USE BY OTHERS

30. The target of the research we conducted, which became the disclosure present in the above referenced patent application, was to devise a series of purification and processing methods to enhance recovery of target proteins from a milk feedstream.

31. The methods provided for are different from any others that I have seen in the literature and represent an enhanced series of methods that allow the identification, separation and processing of bioactive recombinant from the milk of transgenic animals. The molecules so separated are thereafter in condition for further processing for therapeutic use as pharmaceutical compounds or as components of pharmaceutical preparations.

32. Those skilled in the art could readily use the methods taught and techniques presented in the specification and as recited in the current claims of USSN Patent Application 10/635,117.

33. Our research revealed new methods of filtration and processing techniques relevant to the recovery of recombinant proteins from the milk of transgenic animals. As laid out here, the specification of USSN Patent Application 10/635,117 constitutes purification methods different from those presented previously by van Reis et al. The methodologies employed by GTC and various of its researchers in this field have allowed an unprecedented level of control over the purification of target proteins out of milk.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section §1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Dated: 13 Dec 05 By: _____
Mark A. Perreault

Mark A. Perreault

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Education

B.S., Chemistry, with focus on analytical chemistry U.Mass Lowell **1991–1994**

Theory and applications of Chromatography: An advanced course covering the performance of capillary GC, HPLC, modern injection techniques, and detectors.

Employment History

Process and Analytical Development GTC Biotherapeutics. Framingham, MA **2002-Present**
Responsibilities include the development of new filtration processes as they pertain to tangential flow filtration, depth filtration, and virus removal.

Process Development Engineer BD Labware. Bedford, MA **2000-2002**
Responsibilities included the development of new products and processes associated with the labware division. Current products being developed range from micro-arrays to membrane based products.

Research & Development Manager Pall Filtron. Northborough, MA **1994–2000**
Responsibilities included the development of unique polymeric membrane filtration products, implementing improvements to the coating process, and the evaluation of new technologies needed to give us the edge in a competitive market. Additional ongoing responsibilities included designing and executing many types of laboratory experiments. Management activities included supervising day to day R&D experimentation and the coordination of R&D resources with sales / applications support.

Professional Experience

- Joined Filtron Technology Corp. in 1994 and established an analytical lab for the purpose of membrane analysis and development. Developed a new test method for the characterization of Ultrafiltration membranes. Initial accomplishments include the development of novel membrane characterization methods, process development for affinity membranes, and application development for the industrial filtration market.

- Assisted in technology transition to Pall Corp. in 1997 due to company acquisition. Organized and conducted validation studies on three pieces of key equipment in the GMP manufacturing area. Equipment included the casting line, post treatment line, and drying line.

Professional Experience (cont.)

- Developed Pall Filtron's maturing R&D group and responsible for increasing group size and activities two fold. Strengthened communication between product sales groups and development in order to accelerate market ideas. Efficiently managed the R&D lab for over 2 years. Responsibilities included reading and writing project reports, creating a yearly budget, and supervising as many as three fellow scientists.

- Joined BD labware in Febuary 2000 and has taken a leadership role in the engineering development group implimenting state of the art automated processes for the assembly of three different membrane based lab products, increasing capacity by greater than four times.

- Held a key role in BD's new product development team and co-developing products with other biotech research groups. Assisted in the development & optimization of manufacturing many different laboratory products. Sealing techniques used included heat sealing, ultrasonic welding, and adhesive bonding.

- Successfully implemented several new tangential flow filtration techniques in order to increase productivity of various purification processes and initiatiated the exploration of new membranes for the clarification process.

Commonwealth of Massachusetts
County of Middlesex

On this the 13th day of December, 2005, before me, Dawn C. Greenaway, the undersigned Notary Public, personally appeared Mark Perrault, proved to me through satisfactory evidence of identity, which was/were Personal Knowledge to be the person whose name is signed on the preceding document, and acknowledged to me that he signed it voluntarily for its stated purpose.

Dawn C. Greenaway, Notary Public

My Commission Expires:

DAWN COREY GREENAWAY
Notary Public
Commonwealth of Massachusetts
My Commission Expires
February 2, 2012

(Place Notary Seal and/or Stamp Above)